Supplemental Materials

Molecular Biology of the Cell

Hung et al.

A Patient-Derived Cellular Model for Huntington's Disease Reveals Phenotypes at Clinically Relevant CAG Lengths

Claudia Lin-Kar Hung[1], Tamara Maiuri[1], Laura Erin Bowie[1], Ryan Gotesman[1], Susie Son[1], Mina Falcone[1], James Victor Giordano[2,3], Tammy Gillis[2,3], Virginia Mattis[4], Trevor Lau[1], Vickie Kwan[1,5], Vanessa Wheeler[2,3], Jonathan Schertzer[1], Karun Singh[1], *Ray Truant[1]

[1]Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada

[2]Center for Genomic Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

[3]Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

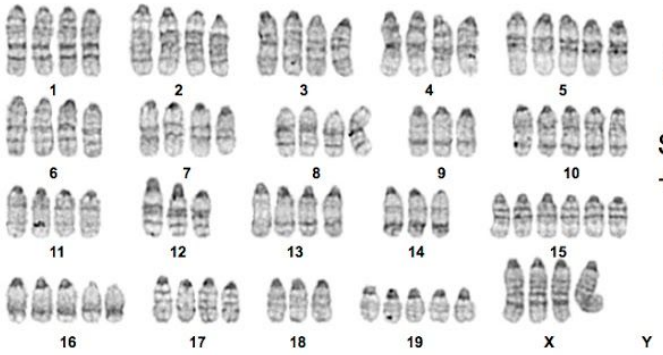
[4]Cedars-Sinai Medical Center, Board of Governors Regenerative Medicine Institute, Los Angeles, CA, United States

[5]Stem Cell and Cancer Research Institute, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada

*Corresponding Author: truantr@mcmaster.ca

Supplemental Figure 1: (A) ST*Hdh*$^{Q111/Q111}$ example karyotype. “mar” denotes marker chromosomes, “+” are additional chromosomes and “-” are missing chromosomes. (B) Senescence-activated beta-galactosidase assay comparing primary-Q21Q18F and TruHD-Q21Q18F cells at ~80% confluency. Senescent cells present blue colour pseudocoloured in greyscale and indicated by black arrow. Scale bar =250μm. (C) TruHD-Q21Q18F plated at lower density (<40% confluency) showed presence of blue colour pseudocoloured in greyscale and indicated by black arrow. Scale bar =250μm. (D) TruHD-Q21Q18F cells at high density that were left to overgrow (100% confluency) showed presence of blue colour pseudocoloured in greyscale and indicated by black arrow, but not as much in TruHD-Q43Q17M. Scale bar =250μm. (E) TruHD-Q21Q18F karyotype of senescent cells showing tetraploidy in 2 of 22 cells analyzed.

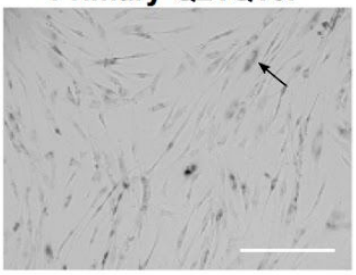
a



ST*Hdh*$^{Q111/Q111}$: 83<4n>,XXXX,+5,-9,
+10,-12,-14,+15,+15,+16,-18,+19,mar

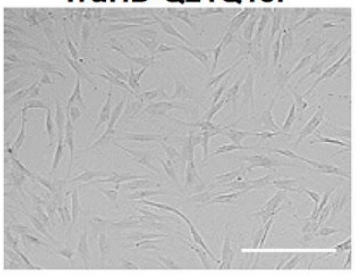
b

Primary-Q21Q18F



Passage 23
10^5/mL

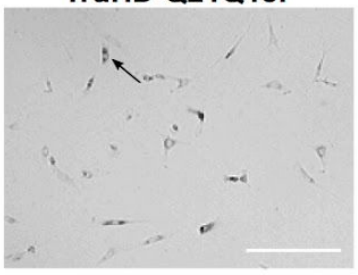
TruHD-Q21Q18F



Passage 49
10^5/mL

c

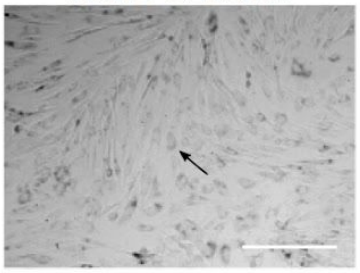
TruHD-Q21Q18F



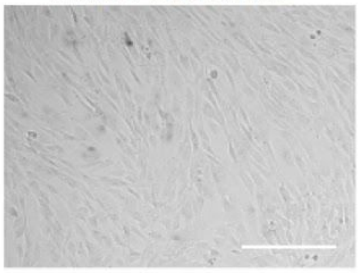
Passage 49
10^4/mL

d

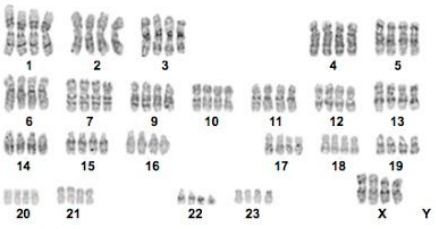
TruHD-Q21Q18F



TruHD-Q43Q17M

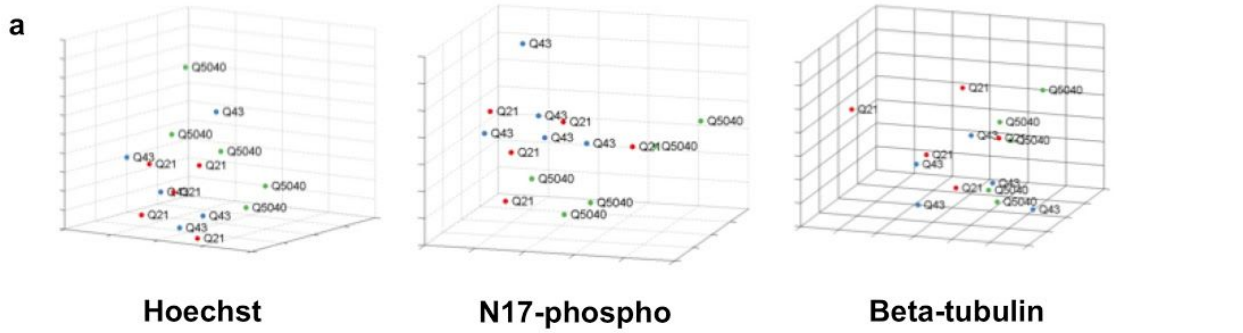


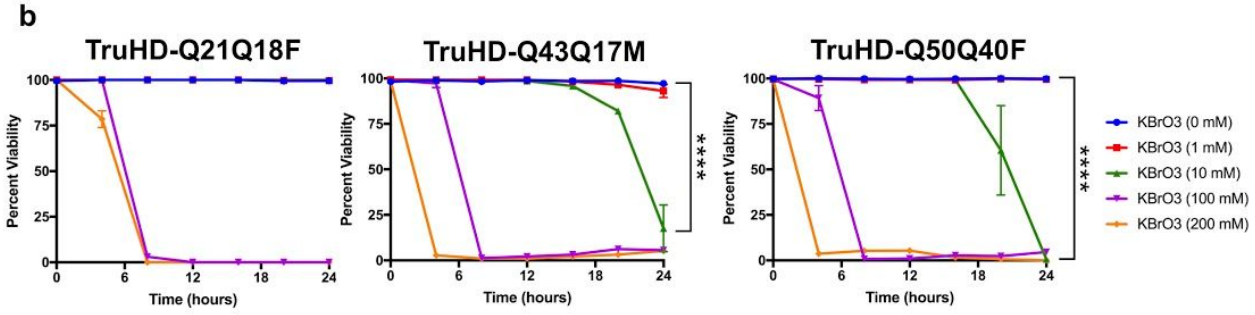
e

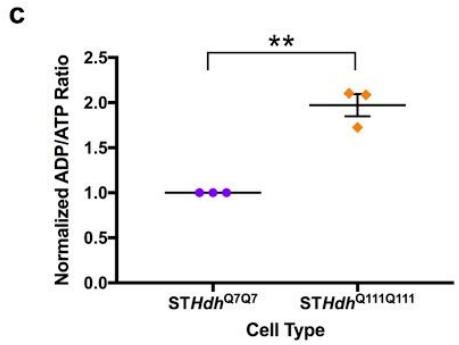


TruHD-Q21Q18F: 92XX (2/22)

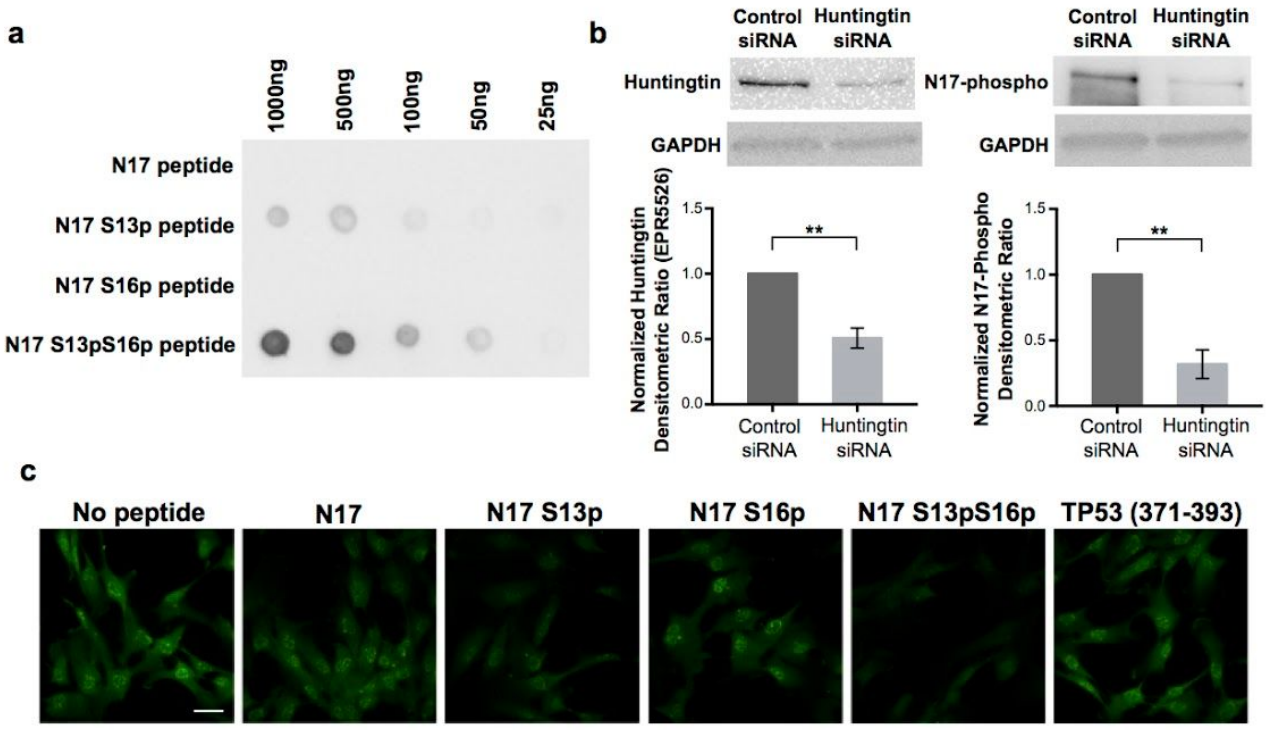
Supplemental Figure 2: (A) Individual PCA plots from Phenoripper comparing images of TruHD cell lines using only Hoechst (405nm), N17-phospho (488nm) and beta-tubulin (640nm) channels. (B) Dose response curves of TruHD cell lines to $KBrO_3$. n=3, N>200. Error bars represent S.E.M. At 24 hours, ****p<0.0001 between 0mM and 10mM treatments in TruHD-Q43Q17M and TruHD-Q50Q40F cells by two-way ANOVA. (C) Normalized ADP/ATP ratio in ST*Hdh* cells at ~75 percent confluency 24 hours after seeding. n=3. Error bars represent S.E.M.**p=0.0014.


a
Q5040
Q43
Q21
Hoechst
N17-phospho
Beta-tubulin

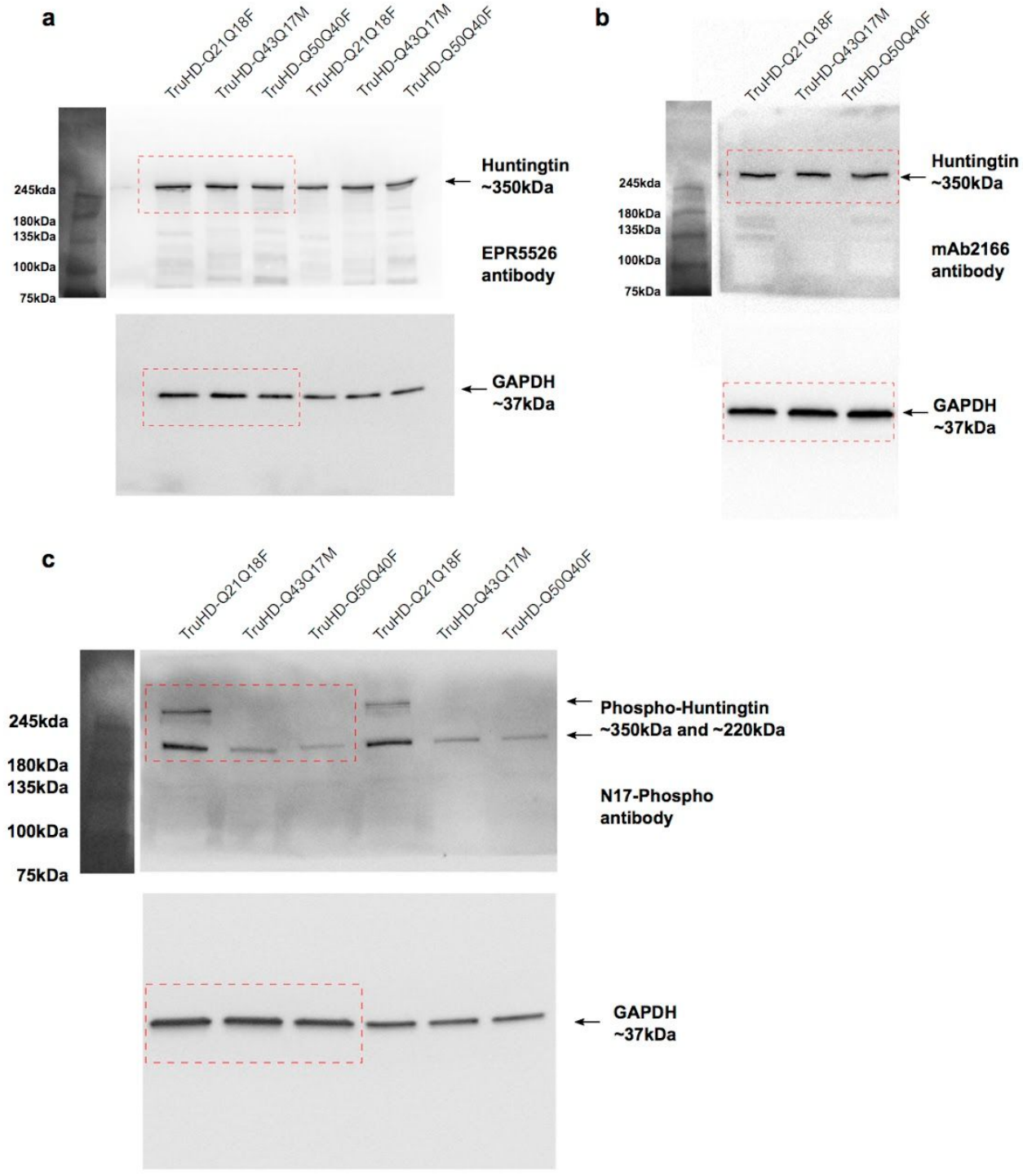

b
TruHD-Q21Q18F
TruHD-Q43Q17M
TruHD-Q50Q40F
Percent Viability
Time (hours)
****
KBrO3 (0 mM)
KBrO3 (1 mM)
KBrO3 (10 mM)
KBrO3 (100 mM)
KBrO3 (200 mM)


c
**
Normalized ADP/ATP Ratio
STHdhQ7Q7
STHdhQ111Q111
Cell Type

Supplemental Figure 3: N17-phospho antibody validation (A) Dot blot with N17 peptides. Unphosphorylated (N17), phosphorylated on serine 13 only (N17 S13p), phosphorylated on serine 16 only (N17 S16p) and phosphorylated on both serines 13 and 16 (N17 S13pS16p). (B) Huntingtin knock-down showing N17-phospho antibody specificity compared to the commercially available total huntingtin antibody EPR5526. Immunoblots were cut horizontally at 75 kDa marker so that GAPDH loading control was probed for separately. **p=0.0035 for N17-phospho and **p=0.0031 for EPR5526. (C) Peptide competition assay with N17 peptides and non-specific TP53 peptide. Scale bar = 20μm.


a
1000ng
500ng
100ng
50ng
25ng
N17 peptide
N17 S13p peptide
N17 S16p peptide
N17 S13pS16p peptide
b
Control siRNA
Huntingtin siRNA
Huntingtin
GAPDH
Normalized Huntingtin Densitometric Ratio (EPR5526)
1.5
1.0
0.5
0.0
**
Control siRNA
Huntingtin siRNA
N17-phospho
GAPDH
Normalized N17-Phospho Densitometric Ratio
1.5
1.0
0.5
0.0
**
Control siRNA
Huntingtin siRNA
c
No peptide
N17
N17 S13p
N17 S16p
N17 S13pS16p
TP53 (371-393)

Supplemental Figure 4: Full huntingtin immunoblots from Figure 3. (A) Huntingtin immunoblot with EPR5526 antibody. Samples were loaded in duplicate. Gel was cut horizontally at 75 kDa marker and blots were probed with EPR5526 or GAPDH antibody. Red, dashed-boxes denotes cropped region used for main figure. (B) Huntingtin immunoblot with mAb2166 antibody. Gel was cut horizontally at 75 kDa marker and blots were probed with mAb2166 or GAPDH antibody. Red, dashed-boxes denotes cropped region used for main figure. (C) Phospho-huntingtin immunoblot with N17-Phospho antibody. Samples were loaded in duplicate. Gel was cut horizontally at 75 kDa marker and blots were probed with EPR5526 or GAPDH antibody. Two main bands were visible (at ~350 kDa and ~220 kDa). Red, dashed-box denotes cropped region used for main figure.


a
TruHD-Q21Q18F
TruHD-Q43Q17M
TruHD-Q50Q40F
TruHD-Q21Q18F
TruHD-Q43Q17M
TruHD-Q50Q40F
245kda
180kDa
135kDa
100kDa
75kDa
Huntingtin
~350kDa
EPR5526
antibody
GAPDH
~37kDa
b
TruHD-Q21Q18F
TruHD-Q43Q17M
TruHD-Q50Q40F
245kda
180kDa
135kDa
100kDa
75kDa
Huntingtin
~350kDa
mAb2166
antibody
GAPDH
~37kDa
c
TruHD-Q21Q18F
TruHD-Q43Q17M
TruHD-Q50Q40F
TruHD-Q21Q18F
TruHD-Q43Q17M
TruHD-Q50Q40F
245kda
180kDa
135kDa
100kDa
75kDa
Phospho-Huntingtin
~350kDa and ~220kDa
N17-Phospho
antibody
GAPDH
~37kDa